Bendamustine Combined with Donor Lymphocytes Infusion in Hodgkin's Lymphoma Relapsing after Allogeneic Hematopoietic Stem Cell Transplantation

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A B S T R A C T

The management of Hodgkin's lymphoma (HL) recurring after allogeneic stem cell transplantation is challenging. We retrospectively describe 18 adults treated with bendamustine followed by escalated donor lymphocyte infusion. Hematological toxicity was manageable (39% grade III to IV neutropenia and 28% grade III to IV thrombocytopenia). The overall response rate was 55%, with 3 complete and 7 partial responses. Median overall and progression-free survival were 11 (range, 1 to 52) and 6 (range, 1 to 28) months, respectively. One-year overall survival of responders (complete or partial) was 70% (95% confidence interval, 42% to 98%), although it was only 16% for nonresponders (n = 8). Our data show that bendamustine followed by donor lymphocyte infusion is feasible and can be efficacious as salvage treatment in HL relapsing after an allograft.

INTRODUCTION

Allogeneic stem cell transplantation (allo-SCT) is a conventional approach for patients with Hodgkin's lymphoma (HL) relapsing or refractory (rel/ref) to autologous hematopoietic stem cell transplantation [1-6]. However, the prognosis of advanced HL for patients undergoing allo-SCT remains poor, with an expected progression-free survival (PFS) ranging from 18% to 39%.

Management of patients relapsing after allo-SCT is not standardized and different approaches, such as reduction of immunosuppression, donor lymphocyte infusion (DLI), a second allogeneic SCT, and new drugs, including brentuximab vedotin, have been reported.

Bendamustine is an active agent in rel/ref HL patients, with a overall response rate (ORR) of 50% to 78% [7-10]. DLI in patients relapsing after allo-SCT induces a response rate (complete plus partial) in the 30% to 50% range and a median duration of 7.5 months [2,11]. Accordingly, we combined bendamustine and DLI in HL patients rel/ref after allo-SCT to synergistically provide reduction of tumor burden by chemotherapy followed by the antilymphoma activity of DLI. So far, this therapeutic option has been reported only in 2 patients [12]. We describe a cohort of 18 HL patients treated with bendamustine and DLI for HL rel/ref after allo-SCT.

PATIENTS AND METHODS

Over an 8-year period (2006 to 2013), we report on 18 adult patients with HL who experienced disease progression (PD) after a reduced-intensity conditioning followed by an unmanipulated allo-SCT and were considered eligible for salvage treatment with bendamustine followed by DLI (Table 1). Patients were treated after inclusion into a compassionate-use program and the provision of written informed consent. The median follow-up from relapse after allo-SCT was 310 days (range, 60 to 2939 days). Donor type was matched related sibling (n = 4), matched unrelated donor (MUD) (n = 2), and mismatched related donor (MMRD) (haploidentical donors n = 9; 7/10 HLA-match, n = 1; 8/10 HLA-match, n = 1; 9/10 HLA-match n = 1). Patients were treated in 2 centers in Milan, Italy: San Raffaele Hospital (patient no. 1 to 8) and Humanitas Cancer Center (patient no. 9 to 18). Patients were considered eligible for bendamustine when they had (1) performance status (Eastern Cooperative Oncology Group) ≤ 2; (2) no immunosoppression ongoing; and (3) no active infection. Patients were considered eligible for DLI after bendamustine in case of (1) absence of active graft-versus-host disease (GVHD), (2) no previous grade III to IV acute GVHD or severe chronic GVHD, and (3) no progression during bendamustine treatment. Bendamustine was administered at the dose of 120 mg/m^2 on days 1 and 2 of 28 days cycles. Eight patients treated at San Raffaele hospital received also rituximab (375 mg/m^2 on day 2 of each cycle) in combination with bendamustine [12-14]. Adequate hematopoietic recovery was required before each cycle (absolute neutrophil count $\geq$1000/μL; platelet count $\geq$75,000/μL) and treatment was delayed or eventually the dose was reduced if these criteria were not met.

Donor lymphocytes were collected by apheresis according to standard center protocol and the desired amount of CD3$^+$ donor T lymphocytes was infused after cytofluorimetric counting without any further manipulation. The initial dose of donor lymphocytes was .5 $\times$ 10^7 CD3$^+$ T cells/kg of recipient's body weight (Humanitas Cancer Center) or 1 $\times$ 10^7 T cells/kg (San Raffaele Hospital), for patients who received a graft from a sibling donor and .5 $\times$ 10^6 CD3$^+$ T cells/kg (Humanitas Cancer Center) or 1 $\times$ 10^6 CD3$^+$ T cells/kg (San Raffaele Hospital) in case of MUD or MMRD. DLI was given 5 to 10 days after each of 2 courses of bendamustine at one-half logarithmic dose escalation. After disease restaging, in the absence of complete remission (CR), limiting toxicities, grade III to IV acute GVHD or severe chronic GVHD, patients were treated with a second course of 2 cycles of bendamustine followed by one-half logarithmic dose escalated DLI up to 5 $\times$ 10^7 CD3$^+$ T cells/kg from a sibling donor or 5 $\times$ 10^6 CD3$^+$ T cells/kg from a MUD or MMRD. Kaplan-Meier estimates of overall survival (OS) were provided for all patients, starting from the first bendamustine cycle. Hazard ratio of mortality was calculated with Cox regression using response as a time-dependent variable.

RESULTS

Median age was 33 years (range, 21 to 48). Ann Arbor-Cotswold stage at relapse was III to IV in 16 of 18 patients.

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Table 1
Patient's Characteristics

Patient No.	Sex/Age, yr	Hystotype, Stage	Disease Status at Allo-SCT	Donor Type, HLA Match	Conditioning Regimen	GVHD Prophylaxis	Time, SCT to Benda, d	No. of Cycles	No. of DLI	T Cell Dose/kg	aGVHD after DLI	cGVHD after DLI	Best Response after Benda [15]	Outcome and time from First Benda to Last Follow-up
# 1	M/41	MC-IV	PR	MUD 10/10	Treo-Flu-ATG	Rapa/MMF	330	1	0				PD	Dead (PD), 14 d
# 2	M/38	NS-IV	SD	MMRD 5/10	Treo-Flu-ATG	Rapa/MMF	525	6	1	1×10^6	IV	None	CR	Alive (PD), 19 mo
# 3	M/37	NS-IV	CR	MMRD 5/10	Treo-Flu-ATG	Rapa/MMF	453	2	0				PD	Dead (PD), 3 mo
# 4	M/44	NS-III	PR	MMRD 5/10	Treo-Flu-ATG	Rapa/MMF	225	4	0				SD	Dead (PD), 4 mo
# 5	F/44	Classic-IV	PR	MMRD 8/10	Treo-Flu-ATG	Rapa/MMF	272	5	2	1×10^6 (I) 5×10^6 (II)	0	Moderate (I) severe (II)	CR	Alive (PR), 40 mo
# 6	F/42	NS-IV	SD	MMRD 5/10	Treo-Flu-ATG	Rapa/MMF	334	6	1	1×10^6	0	Moderate	PR	Dead (infection), 12 mo
# 7	M/28	NS-III	PR	MMRD 9/10	Treo-Flu-ATG -TBI	Rapa/MMF	279	4	0				PR	Alive (CR after brentuximab) 14 mo
# 8	F/46	NS-III	CR	MMRD 5/10	Treo-Flu-ATG	Rapa/MMF	664	4	2	1×10^6 (I) 5×10^6 (II)	0 (I) IV (II)	None (I) NE (II)	CR	Dead (GVHD), 7 mo
# 9	M/29	NS-II	CR	MUD 10/10	Treo-Flu-ATG-TBI	Rapa/MMF	586	4	1	1×10^6	0	moderate	PR	Alive (PR), 12 mo
# 10	M/33	NS-IV	PR	MRD 10/10	Thio-Flu-CTX	CsA/MTX	120	1	1	$.5 \times 10^7$	0	none	PD	Dead (PD), 30 mo
# 11	M/36	NS-IV	PR	MRD 10/10	Thio-Mel-CTX	CsA/MTX	995	7	0				PR	Alive (CR after brentuximab) 45 mo
# 12	M/18	Classic-IV	SD	MRD 10/10	Flu-CTX	CsA	1456	9	3	$.5 \times 10^7$ (I) $.5 \times 10^7$ (II) 1×10^7 (III)	0	none	PR	Alive (CR after II allo-SCT) 53 mo
# 13	M/25	NS-IV	PR	MMRD 7/10	Flu-CTX-TBI	CTX/FK506/MMF	189	1	0				PD	Dead (PD), 5 mo
# 14	M/43	NS-IV	CR	MRD 10/10	Thio-Flu-CTX	CsA/MTX	399	7	3	$.5 \times 10^7$ (I) 1×10^7 (II) 5×10^7 (III)	0	none	PR	Alive (PD), 20 mo
# 15	M/24	NS-IV	CR	MMRD 5/10	Flu-CTX-TBI	CTX/FK506/MMF	613	2	0				PD	Alive (PD), 4 mo
# 16	F/34	MC-IV	CR	MMRD 5/10	Flu-CTX-TBI	CTX/FK506/MMF	133	6	2	$.5 \times 10^6$ (I) 1×10^6 (II)	0 (I) IV (II)	none	PR	Dead (GVHD), 7 mo
# 17	F/20	NS-IV	PR	MMRD 5/10	Flu-CTX-TBI	CTX/CsA/MMF	79	1	0				PD	Dead (PD), 22 d
# 18	M/31	NS-II	PD	MMRD 5/10	Thio-Flu-CTX-TBI	CTX/FK506/MMF	335	2	0				SD	Dead (PD), 12 mo

Benda indicates bendamustine; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; M, male; MC, mixed cellularity; PR, partial response; Treo-Flu-ATG, treosulfan 42 g/m^2, fludarabine 150 mg/m^2, antithymocyte globulin Fresenius (ATG-Fresenius Neovii Biotech, Munich, Germany) 30 mg/kg, rituximab 500 mg; Rapa/MMF, rapamycine, mycophenolate mofetil; NS, nodular sclerosis; SD, stable disease; F, female; Treo-Flu-ATG-TBI, treosulfan 42 g/m^2, fludarabine 90 mg/m^2, antithymocyte globulin Fresenius (ATG-Fresenius Neovii Biotech) 30 mg/kg, total body irradiation 4 Gy; Thio-Flu-CTX, thiotepa 12 mg/kg, fludarabine 60 mg/m^2, cyclophosphamide 60 mg/kg; CsA/MTX, cyclosporin A and methotrexate; Thio-Mel-CTX, thiotepa 10 mg/kg, melphalan 60 mg/m^2, cyclophosphamide 100 mg/kg; Flu-CTX, fludarabine 60 mg/m^2 and cyclophosphamide 60 mg/kg; Flu-CTX-TBI, fludarabine 150 mg/m^2 and cyclophosphamide 30 mg/kg and total body irradiation 2 Gy; CTX/FK506/MMF, cyclophosphamide 50 mg/kg days +3 and +4 and FK506, mycophenolate mofetil; Thio-Flu-CTX-TBI, thiotepa 12 mg/kg and fludarabine 120 mg/m^2 and cyclophosphamide 30 mg/kg and total body irradiation 2 Gy.

Median number of treatment lines before allo-SCT was 4.5 (range, 3 to 13), always including autologous hematopoietic stem cell transplantation. Eight patients had chemorefractory disease. Four patients (patient no. 2, 14, 15, and 18) had already received bendamustine before allo-SCT. Median time to progression after allo-SCT was 7 months (range, 1 to 21). Fourteen patients (78%) had bendamustine as first-line salvage therapy after allo-SCT, whereas 4 had already failed other salvage approach with second allo-SCT, ifosfamide/vinblastine, radiotherapy, vinorelbine/gemcitabine and DLI (patient no. 11), chlorambucil/vinblastine/procarbazine/prednisolone (ChlVPP), bleomycine/methotrexate and DLI (patient no. 15), or radiotherapy and DLI (patients no. 12 and 18). A total of 71 cycles of bendamustine were administered on an outpatient basis with a median of 4 cycles per patient (range, 1 to 9). According to eligibility criteria specified above, only 9 of 18 patients (50%) received DLI. A total of 16 lymphocytes infusions were given, with a median of 2 DLI among eligible patients (range, 1 to 3). A total of 54 adverse events (AEs) occurred after bendamustine administration (44 hematological AEs and 10 nonhematological AEs). Neutropenia grade III to IV developed in 7 patients (39%), thrombocytopenia grade III to IV in 5 patients (28%), and grade III anemia in 2 patients (11%). The most common nonhematologic toxicities were fatigue and nausea (primarily grade I). Four patients experienced serious AE, possibly related to treatment: febrile neutropenia (n = 1), idiopathic pneumonia (n = 2), and cytomegalovirus reactivation (n = 3). Six patients had either delay in cycle timing or dose reduction because of hematological toxicity or concomitant infections. Among 9 patients undergoing DLI, 3 developed acute grade IV GVHD whereas 3 patients developed chronic GVHD (2 moderate and 1 severe). Grade IV acute GVHD was observed in 3 of 4 recipients of haploidentical DLI (patient no. 2 after 1×10^6 $CD3^+$ T cells/kg, patient no. 8 after 1×10^6 $CD3^+$ T cells/kg and 5×10^6 $CD3^+$ T cells/kg, and patient no. 16 after $.5 \times 10^6$ $CD3^+$ T cells/kg and 1×10^6 $CD3^+$ T cells/kg). In total, 4 patients required hospitalization: 1 for supportive therapy of tumor lysis syndrome after bendamustine (patient no. 7) and 3 for acute GVHD.

The ORR was 55% with 3 cases of CR and 7 of partial response, with a median duration of response of 9 months (range, 1 to 26). Median OS and PFS of the entire cohort (n = 18) were 11 months (range, 1 to 52) and 6 months (range, 1 to 28) respectively (Figure 1). One-year OS of responders (complete or partial, n = 10) was 70% (95% confidence interval [CI], 42% to 98%), and 16% (95% CI, 0 to 44%) for nonresponders (n = 8). Cox regression mortality hazard ratio of nonresponders versus responders is 5.94 (95% CI, 1.36 to 25.98), $P = .02$.

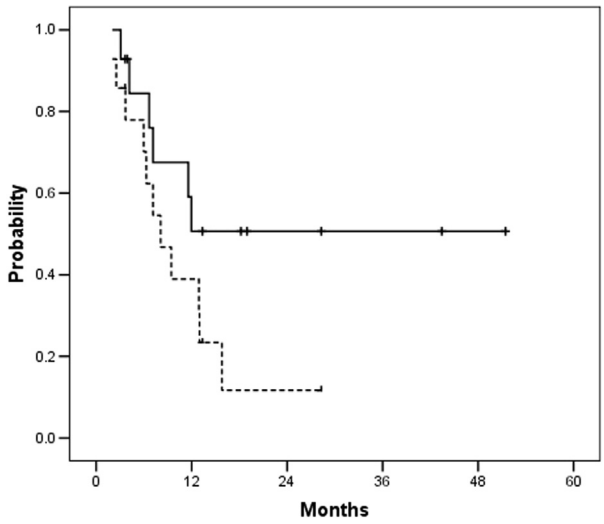

Figure 1. Median OS (solid line) and PFS (broken line) of 18 patients treated with bendamustine +/− DLI for HL relapsed after allografting.

DISCUSSION

Several studies have described the use of bendamustine in heavily pretreated HL patients including patients after autografts [7-10]. A phase II study of 36 rel/ref HL patients treated with bendamustine showed 53% ORR, with 33% CR and a median PFS of 5.2 months [10]. A French study of 28 patients treated with bendamustine for rel/ref HL after autografting showed very similar results, with ORR 50%, 29% CR, and 5.7 months PFS [9]. An Italian study described 41 patients, showing 78% ORR, 29% CR, and 11 months PFS [8]. Recently, a multicenter Italian analysis showed the results of a cohort of 61 rel/ref HL patients rescued with bendamustine—22 of 61 were in PD after an allograft. The reported ORR was 57%, 25% CR, and 10 months PFS [7]. In summary, bendamustine is safe and maintains antilymphoma efficacy, also in rel/ref HL, but it works transiently. Therefore, we hypothesized that bendamustine could promote tumor debulking and induction of tumor immune-exposition in preparation for a consolidation strategy with DLI. Recently, a similar approach has been successfully reported using brentuximab vedotin that selectively induces immunogenic cell death of lymphoma followed by DLI to potentiate the graft-versus-lymphoma effect [16].

In our experience, a cohort of 18 HL who patients relapsed after an allograft treated with bendamustine and DLI showed an antilymphoma effect with a favorable toxicity profile. We observed an ORR of 55% with a 6-month median PFS. One-year OS of responders was 70% versus 16% for non-responders. However, it is difficult to ascertain whether the combination of bendamustine and DLI provided an advantage over single-agent bendamustine. Only prospective controlled studies will indicate the separate roles of bendamustine and DLI in disease control.

In our experience, toxicities in such an advanced clinical setting were manageable and the rate of hospitalization and treatment-related mortality was low. Therefore, these data indicate that the bendamustine-DLI strategy is feasible with an acceptable safety profile.

The intention of the bendamustine and DLI strategy was to achieve chemotherapy-induced tumor debulking followed by the graft-versus-lymphoma effect induced by donor lymphocytes. Based on the assumption that patients already experiencing GVHD have exploited the graft-versus-tumor potential of the SCT immunotherapy [6], DLI was offered only to patients without previous severe GVHD or without active GVHD at relapse. According to this GVHD/graft-versus-lymphoma inducing policy, only one half of patients received DLI after bendamustine administration. The incidence of post-DLI GVHD was high and almost inevitable when a

graft-versus-tumor effect was seen. We observed GVHD in 6 of 9 patients receiving DLI, always when given from an alternative donor (5 from a haplo donor and 1 from a MUD), whereas no GVHD occurred in 3 patients receiving DLI from a matched sibling donor. Nevertheless, PD (and not GVHD) was the main cause of mortality after bendamustine and DLI.

The major limitation of the study is its retrospective design covering an 8-year period, patient selection, as well as the confounding factor of variability of treatment schedules. Given the frail nature of HL in PD after allografting and based on the incidence of bendamustine-related hematological toxicity here reported, a dose of bendamustine of 90 mg/m^2 should probably be probably pursued at first. The DLI dose should be tailored according to the donor source. Based on the high incidence of acute GVHD here reported among recipients of haploidentical allo-SCT, a lower starting dose of 1×10^5 T cells/kg should be considered as a more cautious option in the haploidentical DLI setting.

In conclusion, these practice-based results showing a good feasibility profile and an interesting rate of response in HL relapsing after an allograft suggest that prospective studies in this setting should be pursued.

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